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Abstract
For new techniques to be incorporated into forensic science, they must be compatible with current practices. Here, cyanoacrylate fuming, a common development technique for latent fingerprints, is studied for its compatibility with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) for chemical imaging of latent fingerprints. Half of a fingerprint was fumed while the other half was not; then, the changes in chemical composition and signal intensity were compared with MALDI-MS imaging. No evidence was found that fingerprint compounds are chemically altered by fuming or their signal intensities affected. The only exceptions were significant signal loss for quaternary ammonium compounds from hygiene products and moderate signal loss for tertiary amine compounds. This result is in striking contrast with the previous attempts by others, which is attributed to the difference in instrumentation.

Keywords
forensic science, latent fingerprints, latent fingerprints, cyanoacrylate fuming, fingerprint compounds, matrix-assisted later desorption/ionization, MALDI imaging

Disciplines
Chemistry | Forensic Science and Technology | Radiochemistry

Chemical Imaging of Cyanoacrylate Fumed Fingerprints by Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging

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Running Title: MALDI imaging of cyanoacrylate fumed fingerprints

ABSTRACT

For new techniques to be incorporated into forensic science, they must be compatible with current practices. Here, cyanoacrylate fuming, a common development technique for latent fingerprints, is studied for its compatibility with matrix assisted laser desorption/ ionization mass spectrometry (MALDI-MS) for chemical imaging of latent fingerprints. Half of a fingerprint was fumed while the other half was not; then, the change in chemical compositions and signal intensities were compared with MALDI-MS imaging. No evidence was found that fingerprint compounds are chemically altered by fuming nor their signal intensities affected. The only exceptions were significant signal loss for quaternary ammonium compounds from hygiene products and moderate signal loss for tertiary amine compounds. This result is in striking contrast with the previous attempts by others, which is attributed to the difference in instrumentation.

KEYWORDS

Forensic Science, Latent Fingerprints, Cyanoacrylate Fuming, Fingerprint Compounds, Matrix Assisted Laser Desorption Ionization, MALDI Imaging

Many currently established practices in forensic science lack the technological innovation of other fields. Adoption of recent technological advancements can contribute to the field and help to improve upon the current methods. Analysis of latent fingerprints is one such example. They have been analyzed in a similar way since they were established as a means of personal identification over a century ago. When found in the course of a criminal investigation, latent fingerprints are visualized using a variety of techniques, and the details of the ridge pattern (minutiae) are used to find a database match. The invention of computers has helped to digitalize the process, however, the basic procedure has not changed. Current research aims to establish some new ways to analyze latent fingerprints that can deliver more information about a potential suspect from the chemical composition of their fingerprint. Several classes of compounds have been successfully detected on fingerprints including drugs (both over the counter and illicit) and explosives (1,2). Compounds that are naturally present in fingerprints such as cholesterol, squalene, fatty acids, and lipids have been identified as well (3,4). Many different analytical techniques are now being applied to latent fingerprints for chemical analysis. Mass spectrometry imaging (MSI) has recently emerged as one of the most popular techniques because of its ability to provide a visual image along with chemical information (5,6). In order for these new techniques to be integrated into forensic practice, they need to be tested for compatibility with the current procedures (7).

One of the most common visualization techniques for latent fingerprints is cyanoacrylate fuming. This procedure consists of exposing materials that may have fingerprints on them to cyanoacrylate (superglue) at elevated temperature and humidity in an enclosed chamber. In the presence of cyanoacrylate vapor, a polymer forms on the surface of fingerprint

ridges, making them visible to the eye. Cyanoacrylate fuming is a standard procedure in forensic science so there could be cases where chemical information is needed from a fingerprint that has already been fumed. For such cases, it would be important to know how the fuming process would affect the subsequent MSI experiment.

The advantage of mass spectrometry imaging is the simultaneous collection of both chemical and spatial information; therefore a latent fingerprint can be visualized and distinguished from the surface chemicals unrelated to fingerprints. When fingerprints are visualized using a standard development technique, the imaging capability may not be necessary. However, chemical imaging may be important in cases where standard development techniques are unsuccessful or in cases where chemical information is needed after standard visualization techniques are applied. Regardless of whether or not visualization is done using a development technique such as cyanoacrylate fuming, chemical information from an MSI experiment can be used to gain insight into a suspect.

Imaging mass spectrometers are typically composed of a desorption/ionization source and a mass analyzer. Three ionization sources, matrix assisted laser desorption/ionization (MALDI), desorption electrospray ionization (DESI), and ion beam for secondary ion mass spectrometer (SIMS), have been successfully utilized for fingerprint imaging. Among those, MALDI is by far the most commonly used, often configured with a time-of-flight mass analyzer (TOFMS), because of its versatility and wide availability. Bradshaw et al. have previously analyzed cyanoacrylate fumed latent fingerprints with a MALDI-TOFMS, but with little success. They were unable to identify compounds from the fumed fingerprint spectra, and did not see any of the endogenous compounds usually present in latent prints (7). In addition, Kaplan-

Sandquist et al. have also studied exogenous compounds in fumed fingerprints using MALDI-TOF, but only had an average detection success rate of 18% (8).

In this work, we re-visit the compatibility between cyanoacrylate fuming and MALDI-MSI. We hypothesized that 1) optimal protocol can overcome the previous failure and 2) the particular instrument we use, MALDI-Orbitrap, is superior to MALDI-TOF for the analysis of low mass compounds in complex samples.

Methods

Cyanoacrylate Fuming

Sample slides were taped to the inside of a 2000 mL glass beaker. Two aluminum weigh boats were placed at the bottom of the beaker, one containing water and the other a drop of superglue. The entire beaker was sealed with parafilm and placed on a hot plate. The hot plate was heated to 140-150 °C. Approximately four minutes later, fuming was complete and the sample slides removed.

Sample Preparation

The behavior of endogenous and exogenous compounds was examined in latent fingerprints with and without cyanoacrylate fuming. All experimental methods involving human subjects were approved by our Institutional Review Board. Fingerprint samples for examining endogenous compounds were prepared by rubbing the finger against the forehead and lightly

pressing against a glass slide for several seconds. Preparation of the fingerprint samples for analyzing exogenous compounds involved handling the substances before deposition of the fingerprint. A 1 mg/mL solution of 2,4,6-trinitrotoluene (TNT) in acetonitrile, procaine hydrochloride, and pseudoephedrine hydrochloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Procaine and pseudoephedrine were used to mimic the effects of cocaine and methamphetamine respectively. The solution of TNT was concentrated down to approximately 30 µL and was spotted directly on the finger. Procaine hydrochloride and pseudoephedrine hydrochloride were both powders that could be physically handled before deposition of the fingerprint. Lidocaine is an ingredient in sunburn relief spray, which was purchased from a local retailer. The spray was applied per product instructions before fingerprint deposition.

A glass slide was broken in half and a fingerprint was deposited along the crease so that half of the fingerprint was present on each piece of the slide. One half of the slide was fumed with cyanoacrylate and the other was not. Matrix was applied to both sides of the slide at the same time. They were either sputter coated with silver for five seconds using a 108 Auto sputter coater (Ted Pella Inc., Redding, CA) or sublimated with 2,5-dihydroxybenzoic acid (DHB) using a sublimation apparatus (Chemglass, Wineland, NJ) at 140 °C for 4-5 minutes in a vacuum (<100 mtorr) (9).

Mass Spectrometry Imaging

A MALDI-linear ion trap-Orbitrap mass spectrometer (MALDI-LTQ-Orbitrap Discovery; Thermo Finnigan, San Jose, CA) was used to analyze the fingerprint samples. Modifications

were made to the instrument so it can accommodate an external, frequency tripled 355 nm Nd:YAG laser (UVFQ; Elforlight, Ltd., Daventry, UK) (10). All MSI imaging data was collected using the Orbitrap mass analyzer that has a mass resolution of 30,000 at *m/z* 400. Portions of the fingerprints were chosen for analysis. Data was collected from *m/z* 100-1000 with a 100 µm raster step and a laser spot size of 10-15 µm.

Results and Discussion

Figure 1 describes the typical MADLI-MSI workflow for the chemical imaging of latent fingerprints. First, a fingerprint is deposited on a glass sample slide. The next step is the optional cyanoacrylate fuming. Then, a MALDI matrix (a substance that absorbs the laser energy and aids in desorption and ionization of surface molecules) is applied to the fingerprint. In a MALDI-MSI experiment, a laser beam acts as a sampling probe, analyzing a small area corresponding to the size of the laser spot at a time. The laser beam is rastered across the sample and a mass spectrum is collected at each point. Images are generated by compiling the signal intensities for a specific *m/z* from each mass spectrum collected.

The behavior of endogenous and exogenous compounds was studied in cyanoacrylate fumed fingerprints with several different matrices. The endogenous compounds that were studied include cholesterol, and several fatty acid and triacylglycerol species. Each of these occurred at the same *m/z* value in the fumed fingerprints as they did in the non-fumed fingerprints, indicating that they did not undergo any chemical changes during the fuming process. In addition, signal intensities for the endogenous species were comparable in the mass

spectra of the fumed and non-fumed fingerprints (Figure 2A). Mass spectrometry images obtained from fingerprints that had been cyanoacrylate fumed were of equal quality to those obtained without any development as seen in Figure 2B.

While there was no observed change in the behavior of endogenous compounds due to cyanoacrylate fuming, there was one major difference in the behavior of exogenous compounds. Some of the most common exogenous compounds in fingerprints come from soaps, shampoos, and other hygiene products. These compounds are usually quaternary ammonium derivatives such as distearyldiammonium, behentrimonium, benzyldimethyldodecyl ammonium, and centrimonium. We found that their signals are significantly suppressed after cyanoacrylate fuming compared to other exogenous compounds as shown in Figure 3. However, this signal suppression is not necessarily a disadvantage. As these compounds are present with high signal intensity in the majority of fingerprints, they would not be useful for the differentiation of individuals. With their signal intensity suppressed, it could make other compounds visible that are more useful for individual differentiation. We attribute the signal suppression to the ionic nature of these compounds. As natural cations, they do not need to be ionized in the MALDI source but simple laser-induced desorption is sufficient for mass spectrometry analysis. However, in the presence of anionic acrylate compounds that were detected in the negative mode mass spectra of fumed fingerprints (not shown), they might readily form cation-anion complexes which are neutral and cannot be analyzed by MS, resulting in ion suppression.

To test this hypothesis, we also looked at the behavior of other exogenous compounds: procaine, pseudoephedrine, acetaminophen, trinitrotoluene, and lidocaine. These particular

exogenous compounds were chosen because of their potential forensic interest; procaine and pseudoephedrine are simulants for illicit drugs cocaine and methamphetamine, respectively, acetaminophen is a common over-the-counter drug, trinitrotoluene is a common explosive, and lidocaine is a pain reliever and local anesthetic. As shown in Figure 3, we found that most of these compounds behave similarly to the endogenous compounds that were studied and there is no significant adverse effect on their signal intensities under cyanoacrylate fuming. This trend supports our hypothesis that cationic compounds may be suppressed by anionic acrylate compounds. Upon closer inspection, procaine and lidocaine exhibit some signal suppression, 40% and 34% respectively, clearly beyond the experimental error (~15%). Procaine and lidocaine are both tertiary amines and therefore may be partially present as cationic species in the solid state. Similar to quaternary ammonium compounds, it is possible that protonated tertiary amines may form ion-pairs with acrylate species in the solid state, resulting in suppression of ion signals.

Conclusion

Cyanoacrylate fuming and MALDI-MSI of latent fingerprints are very compatible techniques. Important chemical information can still be gained from a fingerprint after it has been cyanoacrylate fumed without sacrificing signal intensity for most compounds. Significant and moderate signal losses are observed for quaternary ammonium ions and tertiary amines, respectively, which is attributed to ion-paring with acrylate anions. For the endogenous and exogenous compounds studied in this work, we found no evidence of chemical modification

due to the fuming process, so the data analysis is straight forward. Our result is in contradiction to previous efforts (8) that failed to reproducibly obtain MALDI-MS images of fingerprints after cyanoacrylate fuming. We believe this is mostly due to the difference in the instrumentation. MALDI-TOF is operated at high vacuum and produces significant in-source decay for fragile organic compounds, especially when high laser energy is used. With an acrylate polymer covering the fingerprint surface, significant organic contaminations are expected from the in-source decay of this polymer, which would induce ion suppression of fingerprint compounds. However, MALDI-Orbitrap used in this study is operated at medium pressure, 75 mtorr, and has minimum in-source decay (11), with almost no ion suppression. Furthermore, the much higher mass resolution of the Orbitrap allows for the clear distinction of any fingerprint compound from nearby contamination.

We believe that MALDI-MSI could be a valuable tool for forensic science. The chemical information gained from doing MALDI-MSI on a fingerprint could help build a profile of a suspect in cases where the fingerprint does not have a match in any database. Compatibility with traditional forensic techniques such as cyanoacrylate fuming make it more desirable and easier to integrate. As this paper only covers compatibility with one technique, we plan to continue studying the compatibility of MALDI-MSI with other common techniques in forensics, such as powder dusting and lifting of latent fingerprints.

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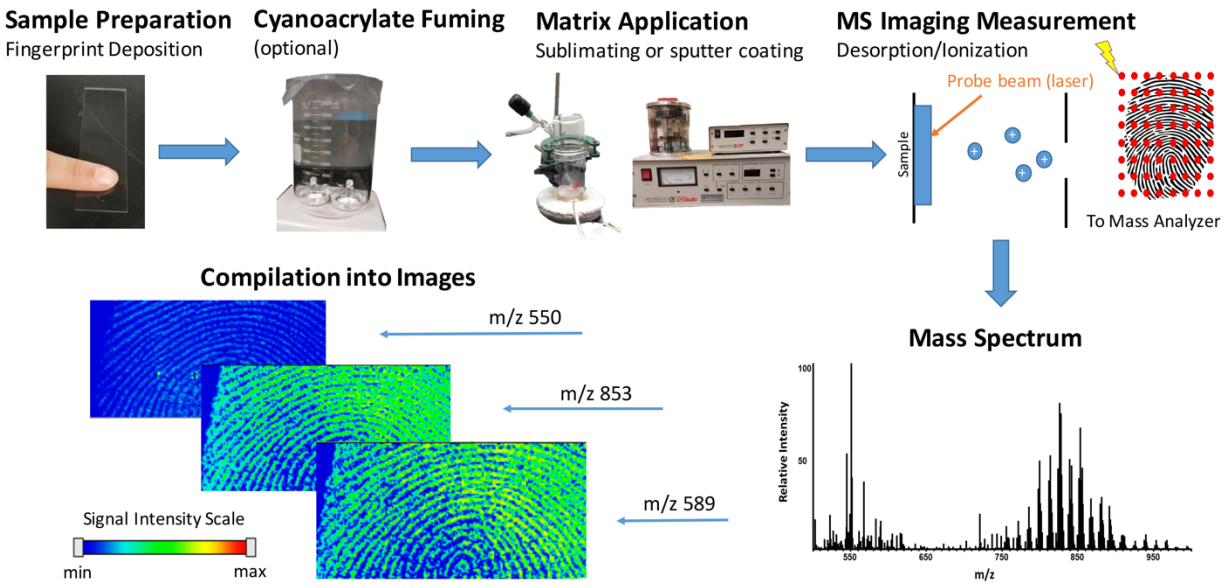
Figure Legends

FIG. 1 MALDI-MSI workflow for data acquisition on fingerprints. Details the steps in the data collection process for a MALDI-MSI experiment on fingerprints. The necessary steps for the experiment include fingerprint deposition, matrix application, mass spectrometry imaging measurement, and image compilation.

FIG. 2 (A) Relative intensities of endogenous compounds in fumed fingerprints normalized to that of non-fumed fingerprints. The error bars correspond to the standard deviations in nine replicates from three individuals. TGs and cholesterol were both analyzed in positive mode with DHB as a matrix, and detected as a sodium adduct, $[M+Na]^+$, and protonated with a water loss, $[M-H_2O+H]^+$, respectively. Fatty acids were analyzed in negative mode with silver as a matrix and was detected as deprotonated, $[M-H]^-$. (B) Mass spectrometry images of three representative compounds with and without fuming: *m/z* 799.68 for TG 46:1; *m/z* 369.35 for cholesterol; *m/z* 253.22 for FA 16:1.

FIG. 3 Relative intensities of neutral and ionic exogenous compounds in fumed fingerprints normalized to that of non-fumed fingerprints. The error bars correspond to the standard deviations in nine replicates from three individuals. All exogenous compounds were analyzed in positive mode with DHB as a matrix, except for TNT and lidocaine, which were analyzed in negative mode and positive mode respectively, both with silver as a matrix. *Cent*., Centrimonium; *BDMDA,* benzyldimethyldodecylammonium; *Behen*., Behentrimonium; *DDA,*

distearyldiammonium; *Acet*., acetaminophen; *TNT,* trinitrotoluene; *Pseud*., pseudoephedrine; *Proc*., procaine; *Lido.,* Lidocaine.



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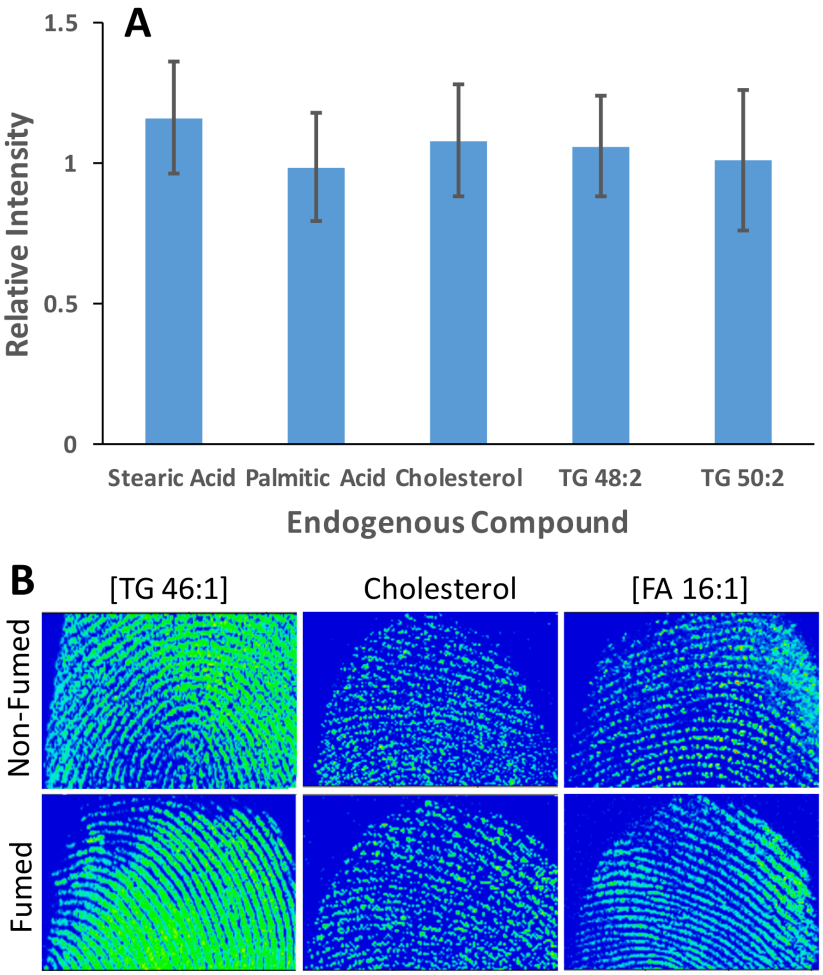

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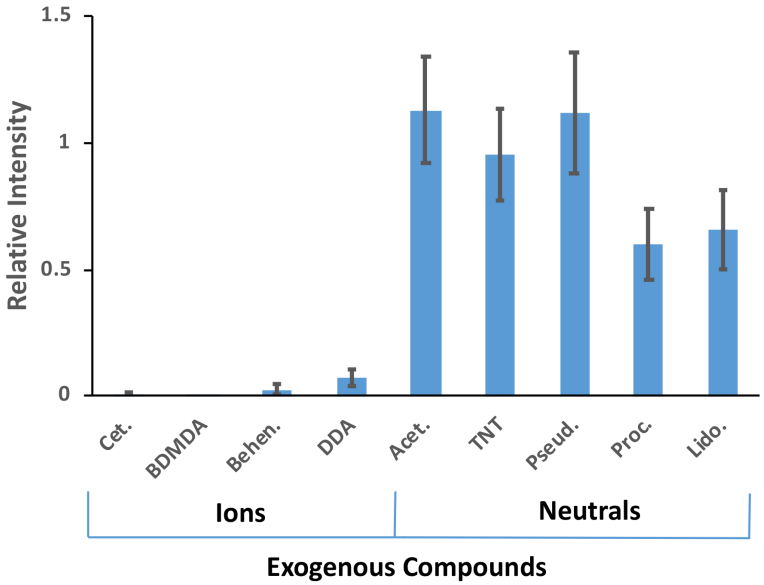

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